Glucagon Receptor–Mediated Extracellular Signal–Regulated Kinase 1/2 Phosphorylation in Rat Mesangial Cells
Role of Protein Kinase A and Phospholipase C

Xiao C. Li, Oscar A. Carretero, Yuan Shao, Jia L. Zhuo

Abstract—Glucagon, a major insulin counterregulatory hormone, binds to specific Gs protein–coupled receptors to activate glycogenolytic and gluconeogenic pathways, causing blood glucose levels to increase. Inappropriate increases in serum glucagon play a critical role in the development of insulin resistance and target organ damage in type 2 diabetes. We tested the hypotheses that: (1) glucagon induces proliferation of rat glomerular mesangial cells through glucagon receptor–activated phosphorylation of mitogen-activated protein kinase extracellular signal–regulated kinase 1/2 (p-ERK 1/2); and (2) this phosphorylation involves activation of cAMP-dependent protein kinase A (PKA) and phospholipase C (PLC)/[Ca2+]i, signaling pathways. In rat mesangial cells, glucagon (1 nM) stimulated [3H]-thymidine incorporation by 96% (P<0.01). This proliferative effect was blocked by the specific glucagon receptor antagonist [Des-His1-Glu9] glucagon (1 μmol/L; P<0.01), a mitogen-activated protein kinase/ERK kinase inhibitor PD98059 (10 μmol/L; P<0.01), a PLC inhibitor U73122 (1 μmol/L; P<0.01), or a PKA inhibitor H-89 (1 μmol/L; P<0.01). The proliferation was associated with a 2-fold increase in p-ERK 1/2 that peaked 5 minutes after glucagon stimulation (P<0.01) and also was blocked by [Des-His1-Glu9] glucagon. Total ERK 1/2 was not affected by glucagon. Pretreating of mesangial cells with U73122 or H89 significantly attenuated ERK 1/2 phosphorylation induced by glucagon. We believe that these are the first data showing that glucagon activates specific receptors to induce ERK 1/2 phosphorylation and thereby increase mesangial cell proliferation and that this effect of glucagon involves both PLC/[Ca2+]i- and cAMP-dependent PKA-activated signaling cascades. (Hypertension. 2006;47[part 2]:580-585.)

Key Words: kidney • cyclic AMP • calcium • diabetes mellitus • glomerulosclerosis • insulin resistance

The pancreatic hormone glucagon is a 29-amino acid peptide that is synthesized in α cells at the periphery of the islets of Langerhans and released primarily in response to low blood glucose levels (hypoglycemia).1,2 Glucagon binds to its specific Gs protein–coupled receptors to activate glycogenolytic and gluconeogenic pathways, causing blood glucose levels to increase.3 The primary action of glucagon is to counter the glucose-lowering effect of insulin, which is synthesized and released by pancreatic β cells.2 Imbalance between the effects of insulin and glucagon on glucose metabolism because of insulin deficiency clearly contributes to the development of type 1 diabetes in humans.3 However, clinical studies have shown that patients with type 2 diabetes do not have insulin deficiency; rather, they exhibit either normal or elevated fasting glucagon levels (hyperglucagonemia) that are unable to decrease appropriately in response to glucose intake.3–5 Thus, increased serum glucagon relative to insulin is responsible for elevated serum glucose levels (hyperglycemia) and, consequently, for increased insulin resistance and development of target organ damage in human type 2 diabetes.3,5,7

Although the role of glucagon in the regulation of blood glucose is well documented, its potential to cause target organ damage in type 2 diabetes remains poorly understood.2,3 In the kidney, glucagon induces glomerular hyperfiltration, a characteristic of early type 2 diabetic glomerular injury.6–9,11 Infusion of glucagon at levels reported in type 2 diabetes increased the glomerular filtration rate (GFR) by ≈50%,8–11 whereas an antibody that neutralizes glucagon decreased GFR in rats with streptozotocin-induced diabetes.12 Persistent glucomer hyperfiltration induced by glucagon may lead to glomerular injury by stimulating growth and proliferation of mesangial cells (MCs) with subsequent mesangial expansion and glomerulosclerosis.13 However, it is not known whether glucagon directly stimulates MC proliferation and activates mitogen-activated protein kinase (MAPK) signaling.

In the present study, we tested the following hypotheses: (1) glucagon induces proliferation of rat MCs through gluca-
glucagon receptor-activated MAPK extracellular signal–regulated kinase (ERK) 1/2 signaling pathways; and (2) glucagon-induced ERK 1/2 signaling involves activation of phospholipase C and cAMP-dependent protein kinase A (PKA). We believe that our data show for the first time that glucagon exerts a proliferative effect on rat glomerular MCs by activating glucagon receptors and inducing MAPK ERK 1/2 phosphorylation and that glucagon-induced MC proliferation and ERK 1/2 phosphorylation require cAMP-dependent PKA- and PLC-activated signaling.

Methods

MC Culture

Rat glomerular MCs were obtained from American Type Culture Collection. The cells were maintained in RPMI-1640 medium (10 mmol/L HEPES, 1 mmol/L sodium pyruvate, 2 mmol/L L-glutamine, 5 mmol/L glucose, and 1500 mg sodium bicarbonate/L; ATCC) containing 12% fetal calf serum (ATCC), penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37°C in 5% CO₂/95% O₂. MCs from passages 6 to 10 were subcultured in 6-well plates containing the above growth medium until they reached 80% confluence and maintained in serum-free medium for 24 hours before the experiment. Unless otherwise specified, all of the experiments were performed in serum-free medium with or without glucagon or other blockers.

Glucagon Receptor Binding and Receptor-Mediated Signaling in MCs

Glucagon receptor binding or mRNA expression has been reported in cultured or microdissected renal tubules. To determine whether glomerular MCs express functional glucagon receptors, subconfluent MCs were incubated with 100 pmol of [125I]-labeled glucagon (specific activity: ~2000 Ci/mmol, Biochem) for 60 minutes at 37°C in a binding buffer containing 50 mmol/L HEPES, 1 mmol/L MgCl₂, 5 mmol/L EGTA, 0.2% BSA, and EDTA-free protease inhibitor mixture (Roche). After incubation, the buffer was removed, and MCs were washed twice with 2 mL ice-cold buffer without BSA. The cells were then collected in tubes and harvested by rapid filtration using a 48-well cell harvester (Brandel). The radioactivity of each sample was measured with a gamma counter. Total glucagon receptor binding was determined with the radioligand alone; for nonspecific binding, we added excess unlabeled glucagon receptor antagonist [Des-His₁-Glu⁰] glucagon (10 μmol/L). Specific binding was calculated as the difference between total and nonspecific binding. To determine glucagon receptor–binding kinetics including affinity constant (Kₐ) and Bₘₐₓ, saturation binding curves and Scatchard plot were performed by incubating MCs with increasing concentrations of [125I]-labeled glucagon (0 to 10 nM) alone or with 100 pmol of [125I]-labeled glucagon in the presence of increasing concentrations of unlabeled glucagon or its antagonist [Des-His₁-Glu⁰] glucagon (0 to 10 μmol/L).

To determine whether glucagon binds to its specific receptors to induce cell signaling, we measured intracellular cAMP accumulation and [Ca²⁺]i mobilization in response to glucagon stimulation. Subconfluent MCs cultured in 6-well plates were treated with vehicle (serum-free medium), glucagon (1 nM), or glucagon plus [Des-His₁-Glu⁰] glucagon (1 μmol/L). The phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (1 mmol/L) was added to all of the samples to prevent cAMP degradation. After treatment, the medium was removed, and the cells were washed and lysed with 0.1 N HCl. The lysates were centrifuged at 1000g, and cAMP levels in supernatants were determined using a sensitive cAMP ELISA kit (R&D).

To determine whether glucagon increases [Ca²⁺]i, by activating PLC, we measured [Ca²⁺]i responses to glucagon (10 nM) in MCs subcultured on coverslips in the absence or presence of [Des-His₁-Glu⁰] glucagon (1 μmol/L) or a PLC-selective inhibitor U73122 (1 μmol/L) using the calcium indicator dye fura 2 (Molecular Probes). MCs grown on coverslips were loaded with fura 2 (2 μmol/L) for 30 minutes at 37°C. After washes, coverslips were mounted on a perfusion chamber maintained at 37°C, which, in turn, was mounted on a Nikon Eclipse TE 2000-U fluorescence microscope coupled with a Lambda DG4 illumination system (Sutter Instruments). Ratiometric calcium responses were continuously recorded at 3-second intervals for up to 10 minutes. The effects of glucagon on [Ca²⁺]i were determined by calculating the average magnitude of the peak [Ca²⁺]i responses during the 200 seconds of glucagon stimulation.

Effects of Glucagon on MC Proliferation

To determine whether glucagon directly stimulates MC proliferation, we measured [³H]-thymidine incorporation, an index of DNA synthesis and cell proliferation. MCs were split into 6-well plates and incubated in RPMI-1640 medium (105/well). Subconfluent MCs were treated with serum-free medium (control; n = 6 wells), glucagon (1 nM, n = 6 wells), glucagon plus [Des-His₁-Glu⁰] glucagon (1 μmol/L, n = 6 wells), glucagon plus PD98059 (10 μmol/L, n = 6 wells), a specific inhibitor of the MAPK/ERK (the upstream kinase of ERK 1/2), glucagon plus U73122 (1 μmol/L, n = 6 wells), or glucagon plus H-89 (1 μmol/L, n = 6 wells), glucagon plus H-89 (1 μmol/L, n = 6 wells). All samples were treated with vehicle mixture (Roche). Protein was measured using a BCA protein assay kit (Pierce). Protein (10 µg) from each sample was separated on 8% to 16% SDS/polyacrylamide gel and transferred semidry onto an Immobilon-P membrane (Millipore). Blots were scanned and analyzed using a microcomputer imaging device (Image Research).

Concentration- and Time-Dependent Effects of Glucagon on ERK 1/2 Phosphorylation in MCs

A agonist-induced phosphorylation of cytoplasmic serine/threonine-containing MAPKs plays an important role in cell differentiation, proliferation, and transformation. To determine whether glucagon induces ERK 1/2 phosphorylation in MCs, subconfluent cells were treated with either serum-free medium or increasing concentrations of glucagon (0.1, 1.0, or 10.0 nM) for 0 minutes to 2 hours. Then, the medium was removed, and MCs were washed twice with ice-cold PBS and lysed with 200 mmol/L NaOH and 0.25% SDS. The contents were transferred to tubes, vacuum filtered, and washed 4 times with 5 mL ethanol/trichloroacetic acid (70%/5%) using a Brandel cell harvester. Incorporated [³H]-thymidine was counted in vials containing 4 mL of scintillation mixture.

Role of Glucagon Receptor–Activated cAMP-Dependent PKA or PLC Signaling in Glucagon-Induced ERK 1/2 Phosphorylation

On binding to specific G protein–coupled receptors, glucagon activates 2 major intracellular signaling pathways, cAMP-dependent PKA and PLC, leading to increased levels of cAMP and [Ca²⁺]. To determine whether these 2 signaling pathways are involved in glucagon-induced ERK 1/2 phosphorylation, subconfluent MCs were treated with glucagon alone (1 nM, n = 6 wells) or pretreated with the potent PKA-selective inhibitor H-89 (1 μmol/L, n = 6 wells) or the potent PLC-selective inhibitor U73122 (1 μmol/L, n = 6 wells) for 30 minutes before glucagon stimulation. Protein samples were extracted for Western blot of total and phosphorylated ERK 1/2.
Statistical Analysis

Where appropriate, results were expressed as mean±SEM. The differences between groups were compared using 1-way ANOVA followed by Dunnett’s post hoc test. P<0.05 was taken as significant.

Results

Glucagon Receptor Binding and Receptor-Activated cAMP and [Ca$^{2+}$], Signaling

Radioreceptor binding assays using $^{125}$I-glucagon showed that MCs express a single class of glucagon receptor binding. Scatchard analysis reveals that MCs bound $^{125}$I-glucagon with an apparent $K_d$ of 32.7 nM (Figure 1A). The $B_{max}$ averaged 1192±40 pmol/mg protein. These binding sites were displaced in a concentration-dependent manner by excess unlabelled glucagon or its antagonist [Des-His1-Glu9] glucagon (10 µmol/L) with a log IC$_{50}$ value of 1.26±0.36 nM and 10.1±0.44 nM, respectively, indicating that they are specific for glucagon (Figure 1A). Exposing MCs to glucagon for 30 minutes led to a 4-fold increase in intracellular cAMP accumulation (control: 15.5±2.8 pmol/mg protein versus glucagon: 68.7±8.6 pmol/mg protein; P<0.01), which was blocked by pretreatment with [Des-His1-Glu9] glucagon (21.0±4.50 pmol/mg protein; P<0.01 versus glucagon; Figure 1B).

We additionally tested whether glucagon increases intracellular [Ca$^{2+}$], by activating PLC-mediated inositol triphosphate (IP$_3$)/[Ca$^{2+}$], signaling. Figure 1C shows representative peak ratiometric calcium responses to glucagon alone and in the presence of the glucagon receptor blocker [Des-His1-Glu9] glucagon or the PLC inhibitor U73122. The averaged peak [Ca$^{2+}$], concentrations in response to glucagon stimulation (n=24 cells) and the effects of glucagon receptor antagonist [Des-His1-Glu9] glucagon (n=16 cells) or the PLC inhibitor U73122 (10 µmol/L, n=18 cells) are shown in Figure 1D.

Glucagon increased [Ca$^{2+}$], 3.5-fold, which was inhibited by [Des-His1-Glu9] glucagon and the PLC inhibitor U73122. Taken together, these results suggest that glucagon binds to functional receptors on MCs and induces cAMP-dependent PKA- and PLC-activated signaling to increase cAMP production and [Ca$^{2+}$], mobilization.

Glucagon Stimulates [H]-Thymidine Incorporation by Activating Glucagon Receptors

We hypothesized that glucagon stimulates MC differentiation by increasing [H]-thymidine incorporation, an index of cell proliferation attributed to increased DNA synthesis. Incubating MCs with glucagon for 24 hours increased [H]-thymidine incorporation by 96% (control: 509±31 cpm/mg protein versus glucagon: 1001±37 cpm/mg protein; P<0.01; Figure 2), and this proliferative effect was blocked by [Des-His1-Glu9] glucagon (633±39 cpm/mg protein; P<0.01 versus glucagon), PD98059 (568±36 cpm/mg protein, P<0.01 versus glucagon), U73122 (603±65 cpm/mg protein, P<0.01 versus glucagon), or H-89 (718±132 cpm/mg protein, P<0.01 versus glucagon). These results suggest that glucagon stimulates MC growth and proliferation via direct activation of specific glucagon receptors and MAPKs, which involves PLC- and cAMP-dependent PKA-activated signaling.

Concentration- and Time-Dependent Effects of Glucagon on MAPK ERK 1/2 Phosphorylation

We tested the hypothesis that glucagon directly activates one of the most important downstream MAPK signaling cascades, ERK 1/2 phosphorylation. Treating MCs with glucagon led to a concentration-dependent increase in ERK 1/2 phosphorylation, reaching a maximum at 10 nM (Figure 3). Based on the concentration-dependent responses, we used 1 nM glucagon for subsequent experiments, because the $K_d$ of the glucagon

Figure 1. Expression of functional glucagon receptors in rat glomerular mesangial cells. (A) $^{125}$I-glucagon radioreceptor binding assays (n=4) showing binding competition by unlabelled glucagon and its receptor antagonist [Des-His1-Glu9] glucagon (10$^{-11}$ to 10$^{-4}$ M), and Scatchard analysis of $B_{max}$ and the apparent $K_d$. (B) Glucagon (1 nM) stimulated intracellular cAMP production, and this effect was blocked by [Des-His1-Glu9] glucagon (1 µmol/L); (C) representative time-dependent peak [Ca$^{2+}$], responses to glucagon stimulation alone (10 nM) and after pretreaments of mesangial cells with [Des-His1-Glu9] glucagon (1 µmol/L) or the PLC inhibitor U73122 (1 µmol/L) for 30 minutes before exposure to glucagon; and (D) quantitative peak [Ca$^{2+}$], responses showing that glucagon-increased [Ca$^{2+}$], was blocked by [Des-His1-Glu9] glucagon (Glu+Ant) and U73122 (Glu+U73122). **P<0.01 vs control; ++P<0.01 vs glucagon.
receptor is reportedly close to this concentration. Glucagon at 1 nM induced rapid ERK 1/2 phosphorylation beginning at 2 minutes (data not shown) and peaking at 5 minutes of stimulation (Figure 4), with the response falling from 30 minutes to 2 hours before returning overnight to a level close to control (data not shown). Total ERK 1/2 was not affected by glucagon. ERK 1/2 phosphorylation was completely blocked by [Des-His1-Glu9] glucagon (Figure 5), suggesting a glucagon receptor–mediated effect.

**Effects of Blocking cAMP-Dependent PKA- and PLC-Activated Signaling on Glucagon-Induced ERK 1/2 Phosphorylation**

Intracellular cAMP-dependent PKA- and PLC-activated [Ca\(^{2+}\)]i signaling pathways are tightly coupled in many cells so that inhibition of one pathway leads to regulation of the other. On binding to its receptors, glucagon couples to Gs proteins to increase intracellular cAMP production and Gi proteins to induce [Ca\(^{2+}\)]i mobilization. In turn, cAMP activates PKA to modulate MAPK, whereas [Ca\(^{2+}\)]i may stimulate MAPK directly. To determine the role(s) of cAMP-dependent PKA or PLC/Ca\(^{2+}\)/Gi–activated signaling in glucagon-stimulated ERK1/2 phosphorylation, we pretreated MCs with H-89 (1 \(\mu\)mol/L), a potent and selective PKA inhibitor, or U73112 (1 \(\mu\)mol/L), a
Glucagon-induced ERK 1/2 phosphorylation was completely blocked by both H-89 and U73122 (Figure 6). These results demonstrate for the first time that rat MCs express functional glucagon receptors, because it was readily displaced by an excess (1 μmol/L) of the glucagon receptor antagonist [Des-His¹-Glu⁹] glucagon. Moreover, we found that glucagon significantly increased intracellular cAMP production and mobilized [Ca²⁺], from intracellular stores, responses that were effectively blocked by [Des-His¹-Glu⁹] glucagon. Taken together, our results suggest that MCs express functional glucagon receptors.

Glucagon has long been implicated in the development of target organ damage in diabetes, because it opposes the actions of insulin by inducing hyperglycemia. Hyperglycemia has been shown to promote cellular growth and proliferation and increase production of extracellular matrix proteins in MCs and vascular smooth muscle cells. The progrowth and proliferative effects of high glucose appear to be mediated by activation of the MAPKs, including ERK 1/2, c-Jun N-terminal kinase, and p38. However, direct effects of glucagon on MC proliferation and activation of ERK 1/2 have not been reported to our knowledge. In the present study, we demonstrated that glucagon directly stimulated [³H]-thymidine incorporation, and this effect was completely blocked by the glucagon receptor blocker completely abolished these effects. Our results are consistent with previous reports that glucagon or glucagon-like peptide 1 activated ERK 1/2 signaling in stable cell lines (HEK 293 cells or pancreatic β cells) expressing the glucagon receptor.

The glucagon receptor represents a family of G protein–coupled receptors, and binding of the agonist to the receptor activates 2 classical signaling pathways, adenylyl cyclase and PLC, leading to increased intracellular cAMP and [Ca²⁺]. In turn, PLC signaling–mediated increase in [Ca²⁺], and diacylglycerol levels activate protein kinase C to induce MAPK phosphorylation. Increased cAMP can also induce MAPK phosphorylation by activating downstream PKA. In HEK 293 cells stably expressing the glucagon receptor, glucagon was shown to activate cAMP-dependent PKA, and depletion of intracellular [Ca²⁺], stores blocked glucagon-induced ERK 1/2 phosphorylation. Our results show that glucagon-induced ERK 1/2 phosphorylation in MCs also requires

Discussion

We believe the present study demonstrates for the first time that rat MCs express specific functional glucagon receptors that, when activated, increase intracellular cAMP accumulation and [Ca²⁺], 2 important signaling pathways for glucagon. It also activates its specific receptors to stimulate MC proliferation by increasing [¹H]-thymidine incorporation, associated with increased MAPK ERK 1/2 phosphorylation.

Glucagon-induced ERK 1/2 phosphorylation was completely blocked by both H-89 and U73122 (Figure 6). These results suggest that cAMP-dependent PKA- and PLC-activated IP/ [Ca²⁺], signaling play an important role in glucagon-induced ERK 1/2 phosphorylation.

Specific glucagon receptor binding or mRNA has been reported in isolated nephron segments, including the proximal tubule, loop of Henle, and cortical collecting duct. Using RT-PCR, Marks et al detected similar levels of glucagon receptor mRNA expression in the glomerulus; however, it is not known whether MCs express functional glucagon receptors. In the renal cortex, the primary effect of glucagon is to increase glomerular filtration. We have shown previously that glucagon-induced glomerular hyperfiltration is comparable to that caused by atrial natriuretic peptide in rats. Glucagon is believed to be responsible for increased GFR after a high-protein meal or the glomerular hyperfiltration seen in early stages of diabetes. Although MCs may, therefore, represent a major target of glucagon in the glomerulus, it remains unclear whether glucagon has a direct effect on MCs or the signaling mechanisms involved. The present study provides evidence that MCs express functional glucagon receptors. Using ¹²⁵I-glucagon radioreceptor binding assays, we found high levels of ¹²⁵I-glucagon receptor binding in MCs (Figure 1A). Binding was considered specific for glucagon, because it was readily displaced by an excess (1 μmol/L) of the glucagon receptor antagonist [Des-His¹-Glu⁹] glucagon. Moreover, we found that glucagon significantly increased intracellular cAMP production and mobilized [Ca²⁺], from intracellular stores, responses that were effectively blocked by [Des-His¹-Glu⁹] glucagon. Taken together, our results suggest that MCs express functional glucagon receptors.

Glucagon has long been implicated in the development of target organ damage in diabetes, because it opposes the actions of insulin by inducing hyperglycemia. Hyperglycemia has been shown to promote cellular growth and proliferation and increase production of extracellular matrix proteins in MCs and vascular smooth muscle cells. The progrowth and proliferative effects of high glucose appear to be mediated by activation of the MAPKs, including ERK 1/2, c-Jun N-terminal kinase, and p38. However, direct effects of glucagon on MC proliferation and activation of ERK 1/2 have not been reported to our knowledge. In the present study, we demonstrated that glucagon directly stimulated [¹H]-thymidine incorporation, and this effect was completely blocked by the glucagon receptor blocker [Des-His¹-Glu⁹] glucagon and the MAPK inhibitor PD98059 (Figure 2). The proliferative effect of glucagon on MCs was likely mediated by glucagon receptor–activated ERK 1/2 signaling. Glucagon induced phosphorylation of ERK 1/2 in MCs in a dose- and time-dependent manner, and pretreating MCs with the glucagon receptor blocker completely abolished these effects. Our results are consistent with previous reports that glucagon or glucagon-like peptide 1 activated ERK 1/2 signaling in stable cell lines (HEK 293 cells or pancreatic β cells) expressing the glucagon receptor.

The glucagon receptor represents a family of G protein-coupled receptors, and binding of the agonist to the receptor activates 2 classical signaling pathways, adenylyl cyclase and PLC, leading to increased intracellular cAMP and [Ca²⁺]. In turn, PLC signaling–mediated increase in [Ca²⁺], and diacylglycerol levels activate protein kinase C to induce MAPK phosphorylation. Increased cAMP can also induce MAPK phosphorylation by activating downstream PKA. In HEK 293 cells stably expressing the glucagon receptor, glucagon was shown to activate cAMP-dependent PKA, and depletion of intracellular [Ca²⁺], stores blocked glucagon-induced ERK 1/2 phosphorylation. Our results show that glucagon-induced ERK 1/2 phosphorylation in MCs also requires
receptor-mediated activation of cAMP-dependent PKA and PLC/[Ca^{2+}]_i-mediated signaling. Pretreatment of MCs with H-89, a potent and selective cAMP-dependent PKA inhibitor, or U73122, a potent and selective PLC inhibitor, completely abolished glucagon-stimulated [3H]-thymidine incorporation or ERK 1/2 activation. Because glucagon can directly activate its receptors to increase intracellular cAMP production and [Ca^{2+}]_i in MCs, these results support our hypothesis that cAMP-dependent PKA and PLC/[Ca^{2+}], signaling pathways play an important role in glucagon-induced MC proliferation and MAPK ERK 1/2 activation.

**Perspectives**

We believe that the present study demonstrates for the first time that glucagon activated specific receptors to increase MC proliferation by stimulating MAPK ERK1/2 phosphorylation and that these effects of glucagon are dependent on activation of PLC/[Ca^{2+}]_i and cAMP/PKA-mediated signaling cascades. Together with the well-documented effects of glucagon on glomerular hyperfiltration, direct growth and proliferative effects of glucagon on MCs and underlying signaling mechanisms may have important clinical implications in the development and treatment of type 2 diabetic glomerular injury. Because plasma insulin levels remain relatively normal, whereas plasma glucagon levels are inappropriately elevated in type 2 diabetes, our results support the concept that pharmacological blockers or molecular intervention targeting glucagon and/or its receptors may be a useful approach in the clinical prevention and treatment of target organ damage in type 2 diabetes.

**Acknowledgments**

The work was supported in part by the National Institute of Diabetes, Digestive and Kidney Diseases (RO1DK067299; to J.L.Z.) and Henry Ford Health System institutional grant (A10217; to J.L.Z.). O.A.C. is supported by a National Heart, Lung, and Blood Institute program project grant (HL28982).

**References**


Glucagon Receptor–Mediated Extracellular Signal–Regulated Kinase 1/2 Phosphorylation in Rat Mesangial Cells: Role of Protein Kinase A and Phospholipase C

Xiao C. Li, Oscar A. Carretero, Yuan Shao and Jia L. Zhuo

Hypertension. 2006;47:580-585; originally published online January 3, 2006;
doi: 10.1161/01.HYP.0000197946.81754.0a

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/47/3/580

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/